

## On Certain Thaliacea (Tunicata) from the Pacific Ocean, with Descriptions of Two New Species of Doliolids<sup>1</sup>

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THE MATERIAL upon which this paper is based has been taken from the extensive plankton collections available at the Scripps Institution of Oceanography. The samples examined were from three sources: the Shellback Expedition to the area off Central and South America in 1952, the Mid-Pacific Expedition to the Marshall Islands area in 1950, and from the routine Marine Life Research Program sampling off the west coast of the United States and Baja California, Mexico. The two expeditions were in part supported by grants from the Office of Naval Research. The Marine Life Research Program is the Scripps Institution's component of the California Cooperative Oceanic Fisheries Investigations, a project sponsored by the Marine Research Committee of California. The work has been further supported by a postdoctoral grant from the Rockefeller Foundation for the senior author.

Two new doliolids found in the plankton samples collected on the Shellback Expedition have already been described (Tokiooka and Berner, 1957), and further examination of the samples has yielded two additional new doliolids from the same area.

In addition to the descriptions of the two new species, both of which belong to the genus *Doliolina*, additional notes are given on

*Doliolina undulata* Tokiooka and Berner 1957, *Doliopsoides horizonsi* Tokiooka and Berner 1957, *Doliolina intermedium* (Neumann) (1906), *Cyclosalpa strongylenteron* Berner 1955, and *Cyclosalpa bakeri* Ritter 1905.

### *Doliolina obscura* new species

Fig. 1A-E

Many specimens of this species were found at Shellback stations 137, 142, 145, 155, 160, and 180. The species also occurs quite often in the samples collected by the Marine Life Research Program, although it is never numerous.

The gonozooids may be somewhat stumpy in outline; they are usually less than 5 mm. long and may range up to 4 mm. in body diameter. The test is of moderate thickness, is rather soft, and may be easily stripped from the body. When found with the test in place the specimens tend to be covered with detrital material from the sample.

Muscles I, VII, and VIII are narrower than muscles II-VI. Muscle VII is distinctly interrupted at the mid-ventral line. The sixth and seventh intermuscular zones, especially the latter, are narrower than the others.

The endostyle (*ed.*) is long, extending anteriorly at least to three-fourths of the second intermuscular zone and posteriorly to the anterior margin of muscle V. The anterior margin of the peripharyngeal band (*p.b.*) extends nearly to muscle I. The ciliated groove (*c.g.*) is situated in the middle of the second intermuscular zone. The dorsal ganglion (*d.g.*) is

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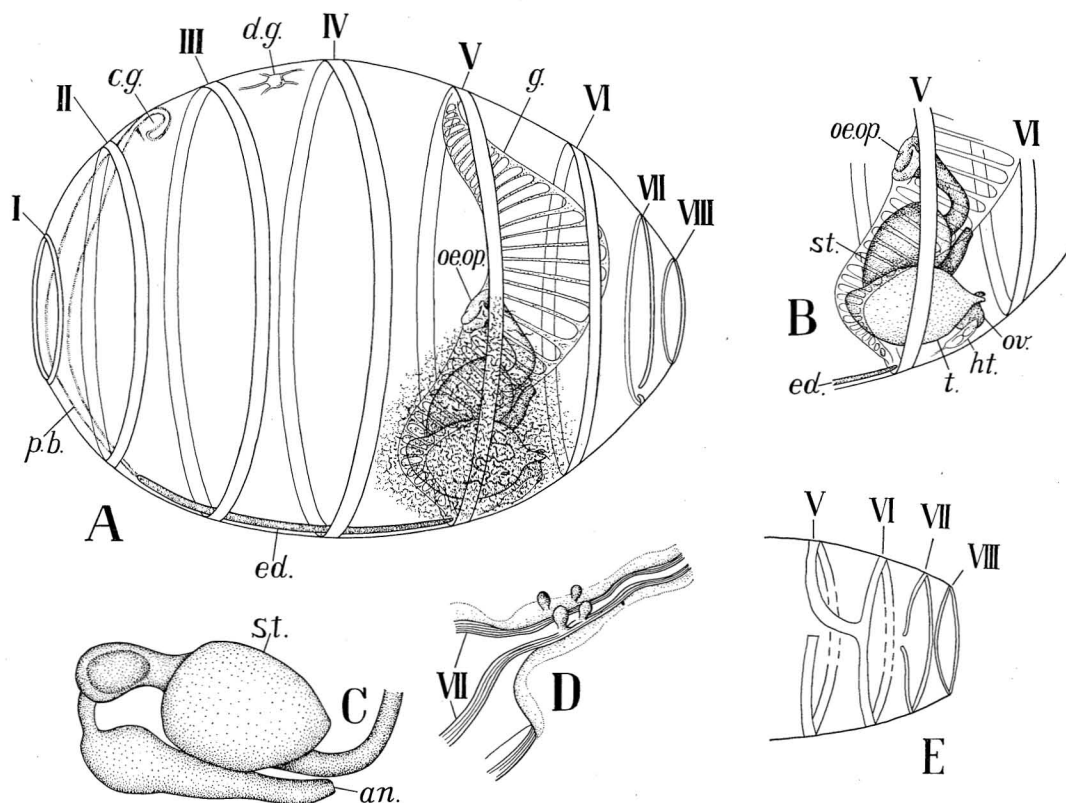


FIG. 1. *Doliolina obscura* n. sp. A, Animal from left side: a 5 mm. gonozooid. B, Alimentary organs and gonads from left side. C, Alimentary canal. D, Ventral protuberance, phorozooid. E, Aberrant form showing arrangement of muscles viewed ventrally.

Abbreviations: I-VIII, body muscles; *an.*, anus; *c.g.*, ciliated groove; *d.g.*, dorsal ganglion; *ed.*, endostyle; *g.*, gill septum; *ht.*, heart; *int.*, intestine; *oe.op.*, oesophageal opening; *ov.*, ovary; *pb.*, peripharyngeal band; *st.*, stomach; *t.*, testis; *v.d.*, vas deferens.

situated in the third intermuscular zone with its posterior edge at the center of the zone. The gill septum (*g.*) is sigmoid in shape extending from the dorsal to ventral position of muscle V, (Fig. 1*a*). At the lower flexure the anterior margin reaches slightly beyond the middle of the fourth intermuscular zone. At the upper flexure the posterior margin extends slightly beyond the posterior margin of muscle VI. The median crest of the gill septum is slightly displaced to the right side in the ventral half of its course, where the massive alimentary organs and gonads are situated. There are about 40 elongate stigmata.

The alimentary organs and gonads are surrounded by densely aggregated corpuscles which make it very difficult to examine the structure of the organs closely. These corpuscles are probably reddish orange when the animal is alive. Staining of the specimens with Rose Bengal makes it somewhat easier to study this portion of the body.

The oesophageal opening (*oe. op.*) is located near the center of the circle described by muscle V. The stomach (*st.*) is located on the sagittal plane of the body and is roughly oval in outline. The intestine forms a simple loop and ends with the anus (*an.*) at a posi-

tion slightly behind the cardiac end of the stomach. The hind stomach is distinct and there is an obvious constriction between it and the following portions of the gut.

The ovary (*ov.*) is located near the middle of the fifth intermuscular zone or slightly posterior to it. The testis (*t.*) is a fusiform-shaped stumpy mass, oriented anteroposteriorly, and extending from the ovary to near the middle of the fourth intermuscular zone. The heart is located near the ovary.

The phorozoid is similar in appearance to the gonozoid, though the corpuscular aggregation over the area of the gut tends to be reduced in the phorozoid. Both the ventral ends of muscle VII extend into the ventral protuberance (Fig. 1D).

At a glance this new species closely resembles *D. mülleri* (Krohn 1852). It differs from the latter by having the ventral interruption of muscle VII and in the anterior-posterior position of the testis. In *D. mülleri* the testis is situated somewhat dorsoventrally and muscle VII forms a complete loop. The sigmoid curve of the gill resembles *D. sigmoides* Garstang 1933 (= *Doliolum krohni* Neumann 1906). The new species completely lacks the epidermal tentaclelike processes characteristic of *D. sigmoides*. Interrupted muscle VII is another differentiating characteristic. The new species differs from *D. undulata* in the shape of the stomach and in the shape and location of the testis. In addition, the aggregation of corpuscles around the alimentary organs and the gonads is unique. The species name comes from this aggregation which makes these organs obscure.

The specimens upon which this description is in part based are deposited in the U.S. National Museum under the following numbers:

HOLOTYPE: U.S.N.M. no. 11371, *Doliolina obscura*, gonozoid, 1 specimen from Shellback station 137 located at 13° 43.5' S. 81° 08.5' W.

PARATYPES: U.S.N.M. no. 11372, gonozoids, 10 specimens, *Doliolina obscura* from Shellback station 137.

From the MLR material one aberrant form was taken in which the fifth body muscle was interrupted and the left limb of that muscle extended back to connect with muscle VI. In other respects this specimen did not differ from the species. The muscle arrangement is shown in Figure 1E.

### *Doliolina separata* new species

Fig. 2

A few specimens of this diminutive doliolid were found in the samples from Shellback stations 137, 145, and 180. Most of them were poorly preserved and immature, but three mature, fairly well-preserved specimens were found at station 137. The following description is based on these three specimens.

The specimens were 1.5, 1.7, and 1.7 mm. long. The body is somewhat elongate. The test is of moderate thickness and is easily stripped from the body. Under the microscope many minute cells can be observed on the surface of the test.

Body muscles I, VII, and VIII are very narrow. Muscle II is somewhat narrower than muscles III-VI. Muscle VII is clearly interrupted at the mid-ventral line. The first and seventh intermuscular zones are narrower than the others.

The endostyle (*ed.*) is very long, extending from the posterior margin of muscle II to near the anterior margin of muscle V. The anterior border of the peripharyngeal band (*p.b.*) closely approaches muscle I. The ciliated groove (*c.g.*) is situated approximately in the middle of the second intermuscular zone. The dorsal ganglion (*d.g.*) is located in the third intermuscular zone near the posterior margin of muscle III. The gill septum (*g.*) extends from muscle VI dorsally to muscle V ventrally, and bears 10 pairs of somewhat elongate stigmata.

The oesophageal opening is located at the center of the circle formed by muscle VI. The whole alimentary canal forms an S-shaped loop towards the anteroventral side, in the

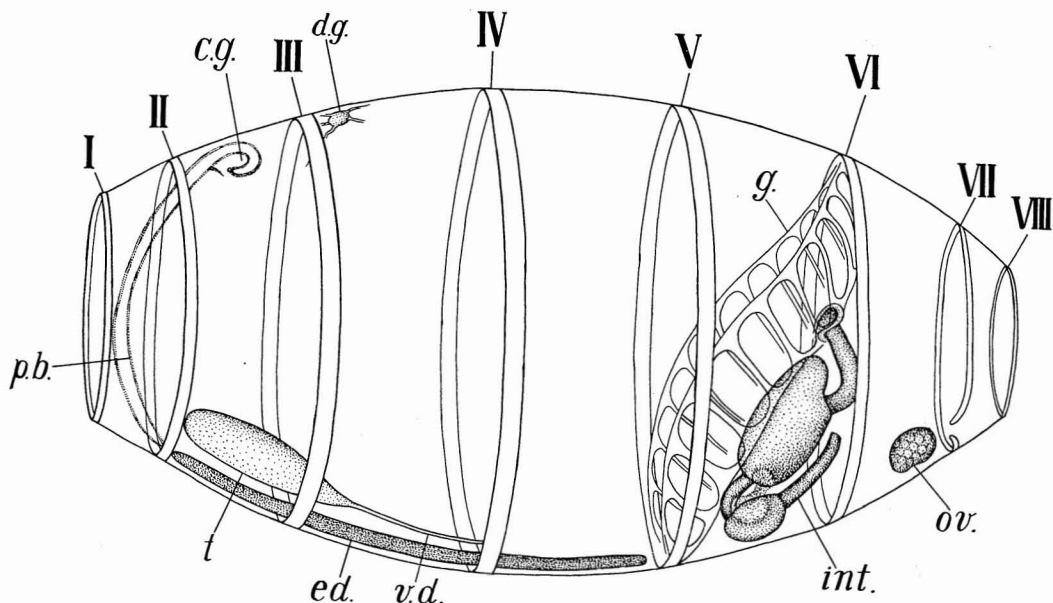


FIG. 2. *Doliolina separata* n. sp., a 1.7 mm. gonozooid from left side.

sagittal plane of the body. The stomach is roughly an elongate ellipse in outline with its cardiac portion prominently protruded. The anus opens near the cardiac end of the stomach. The anterior margin of the intestinal loop (*int.*) extends to near the posterior margin of muscle V. The mid-intestinal region is swollen in some specimens. Usually there is a small group of cells on each side of the intestinal loop, in some cases these groups may consist of two parts, one large and one small.

The ovary (*ov.*) is situated ventrally near the middle of the sixth intermuscular zone. The testis (*t.*) is a club-shaped organ extending from the posterior margin of muscle II to slightly beyond the posterior margin of muscle III. The vas deferens (*v.d.*) opens into the pharynx at the level of muscle IV.

The phorozooid is quite similar to the gonozooid. It lacks gonads and has a ventral protuberance, into which the ends of muscle VII project.

*Doliolina separata* n. sp. resembles *D. krohni* (Herdman 1888) and *D. indicum* (Neumann 1906) in that it has an obliquely stretched gill

septum. The shape of the testis is somewhat reminiscent of *D. intermedium* (Neumann 1906). The ventral interruption of muscle VII and the peculiar arrangement of the gonads, in which the ovary and testis are widely separated from each other, is unique and this latter characteristic is the basis for the specific name here given.

The material upon which this description is in part based is deposited in the U.S. National Museum:

SYNTYPES: U.S.N.M. no. 11373, 2 specimens, Gonozooid, *Doliolina separata* from Shellback station 137.

ALLOTYPES: U.S.N.M. no. 11374, 4 specimens, Phorozooid, *Doliolina separata* from Shellback station 137.

### *Doliolina intermedia* (Neumann 1906)

Fig. 3

*Doliolum intermedium* Neumann, 1906: 211–212.

Many specimens of *D. intermedium* have been found in the Shellback and the Mid-

Pacific material. They have an average length of about 10 mm. The test is very soft, of moderate thickness, and is easily stripped from the animal. All eight body muscles are complete. Muscles VII and VIII are very narrow and muscle VI is somewhat narrower than muscles I-V. The third intermuscular zone is the widest and the seventh is the narrowest.

The endostyle (*ed.*) extends anteriorly to beyond the second intermuscular zone and posteriorly to the middle of the fourth zone. The anterior margin of the peripharyngeal band (*p.b.*) approaches the posterior margin of muscle I. The ciliated groove (*c.g.*) is situated in the center of the second intermuscular zone. The dorsal ganglion (*d.g.*) is located in the third intermuscular zone near the posterior margin of muscle III. The gill septum (*g.*) attaches at muscle IV dorsally at the middle, or slightly beyond, the fourth intermuscular zone ventrally. It bears about 50 pairs of elongate stigmata.

The loop of the alimentary canal is situated

vertically in the fifth intermuscular zone with the oesophageal opening (*oe. op.*) on the sagittal plane. The stomach is elongate and rectangular in outline with the anterior margin slightly convex. The hind-stomach is very distinct with an extreme constriction between it and the intestine. The anus is located near the cardiac end of the stomach.

The ovary (*ov.*) is situated just in front of muscle VII. The testis (*t.*) projects anteriorly along the left side of the endostyle with its anterior portion often curving dorsally along the posterior side of the peripharyngeal band.

***Doliolina undulata* Tokioka and Berner 1958**

Fig. 4

*Doliolina undulata* Tokioka and Berner, Pacific Sci. 12(2):135-138.

Several more specimens of *D. undulata* have been found in the material from the Shellback Expedition. In some of these, most frequently small mature specimens, the testis is stretched obliquely between muscles V and VI with

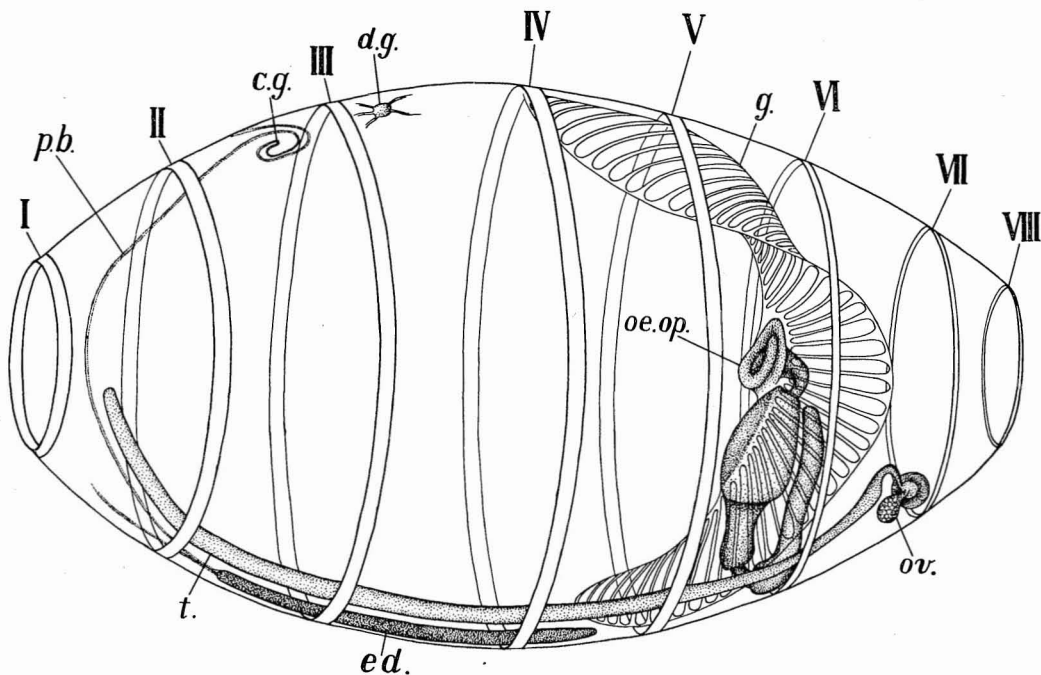


FIG. 3. *Doliolina intermedia* (Neumann) 1906, a 10 mm. gonozooid from left side.

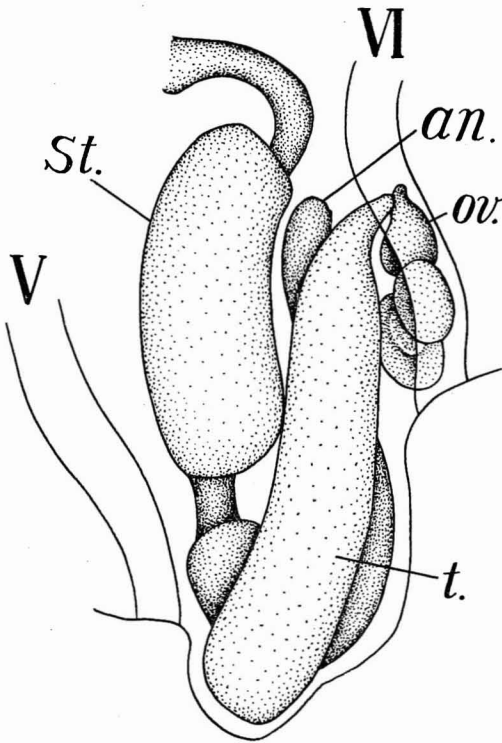


FIG. 4. *Doliolina undulata* Tokioka and Berner 1958, alimentary organs and gonads, from left side.

very little undulation. In these cases the ventral part of the body, just posterior to muscle V, is distended by the testis. The appearance of the testis and its relative position against the intestinal loop are fundamentally the same as in specimens with the undulating testis.

#### Subdivisions of the genus DOLIOLINA

With the descriptions of *D. undulata* Tokioka and Berner (1958) and here of *D. obscura* and *D. separata*, we find two types of muscle pattern within the genus *Doliolina*. These patterns, one in which all of the muscles form complete loops and one in which the seventh muscle is interrupted ventrally, distinctly divide the genus into two groups. On this basis we have established the following subdivision of the genus:

1) *Doliolina perfecta*: The muscle bands of this group all form complete bands. The following species are included: *indica* (Neumann

1906), *intermedia* (Neumann 1906), *resistible* (Neumann 1913), *mülleri* (Krohn 1852), *krohni* (Herdmann 1888), and *sigmoides* Garstang 1933.

2) *Doliolina imperfecta*: Within this group the seventh muscle band is always interrupted at the mid-ventral line. The species in this group are known only from the eastern Pacific Ocean, and include: *undulata* Tokioka and Berner 1958, *separata* n. sp., and *obscura* n. sp.

The shape and position of the gonad and the arrangement of the gill septum vary considerably within the two groups, and in some cases convergence of these features may be seen. We thus hesitate to treat them as subgenera of *Doliolina* but feel the problem may better be settled by more extensive collections and further critical examination of the respective species.

The discovery of three species of doliolids, in addition to the one previously known (*Dolioletta mirabilis*), in which a body muscle is interrupted gives support to the argument for the abandonment of the ordinal name Cyclomyaria for the doliolids, in favor of the name Doliolida. This change has been generally accepted since Garstang's review (1933).

#### *Doliopsoides horizoni* Tokioka and Berner 1958

Figs. 5A, B

Many additional specimens of *D. horizoni* have been found in the material from the Shellback Expedition. In some of these the alimentary organs are better preserved than in any of the previous specimens. Close examination of these organs reveals that the stomach is provided with several fingerlike protuberances. In one especially well-preserved specimen, four pairs of these protuberances were found on the ventral, posterior portion of the cardiac stomach and two pairs on the ventral, posterior portion of the pyloric stomach. The number of protuberances is not constant among the specimens.



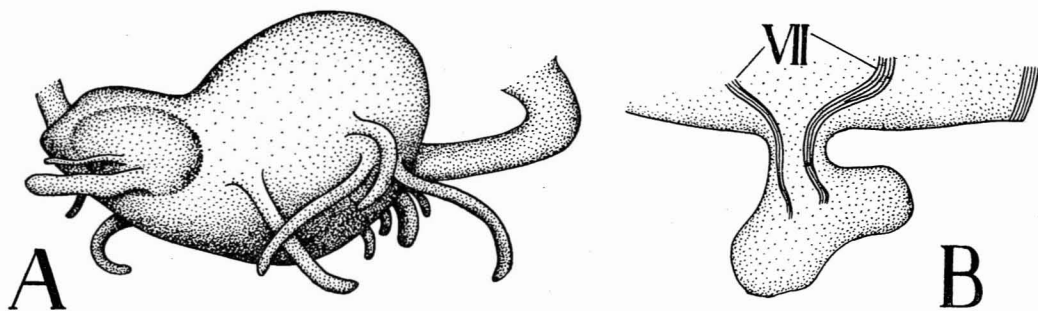


FIG. 5. A, *Doliopsoides horizoni* Tokioka and Berner 1958, stomach showing protuberances. B, *Doliopsoides horizoni*, ventral protuberance of phorozooid.

One individual of the phorozooid generation was found among the specimens. It has a short ventral protuberance into which both ends of muscle VII project.

#### COMPARISON OF ALIMENTARY REGION OF *Cyclosalpa strongylenteron* AND *C. bakeri*

In the examination of aggregate individuals of *Cyclosalpa strongylenteron* Berner 1955, some distinct asymmetries were found in the arrangement of the alimentary organs and gonads. These features differed somewhat between the dextral and sinistral individuals, although the musculature showed almost complete symmetry. The features of the alimentary organs and the gonads are compared with those of the aggregate form of *C. bakeri* Ritter 1905, whose visceral portion closely resembles that of *C. strongylenteron*.

In *Cyclosalpa strongylenteron* Berner 1955 (Figs. 6A, B, 7) the dextral individuals are those derived from the right side of the stolon when it is placed so that the stolo-individuals are situated dorsal side up, with the posterior end toward the right and with the proximal end of the stolon pointing away from the observer. The sinistral individuals are those derived from the left side of the stolon.

In the dextral individuals the intestinal loop is U-shaped. The anus (*an.*) opens on the left side at a level slightly anterior to the oesophageal opening (*oe. op.*). There is a large elongate blind sac or caecum (*l.c.*) attached to the

ventral side of the lower branch of the loop, about one third of the way between the oesophageal opening and the posterior margin of the loop. The tip of the caecum reaches posteriorly nearly to the posterior margin of the gut loop but seldom beyond it. A small sac or protuberance (*r.c.*) is found on the left wall of the alimentary tract just dorsal to the base of the large caecum (Fig. 7). The duct of the circum-intestinal gland (*d.c.g.*) opens into the tip of this small sac, which could be considered merely a swelling of the proximal end of the duct. It is clear, however, that it is actually a projection of the alimentary tract. The swollen portion is stained reddish orange by Rose Bengal, like the alimentary tract, rather than purplish red like the duct. The relative positions of the small and large sacs indicate that they are the right and left caeca respectively.

The testis (*t.*) is about half the length of the left caecum and is situated on the right side of the ventral branch of the intestinal loop near its posterior margin. The vas deferens (*v.d.*) crosses the duct of the circum-intestinal gland on the left side and opens into the atrial cavity just in front of the point where the dorsal visceral muscles (*l.x.* and *r.x.*) from each side unite. These joined muscles project posteriorly into the center of the intestinal loop passing to the right of both the vas deferens and the duct of the circum-intestinal gland. The left visceral muscle (*l.x.*) runs along the ventral side of the intestinal loop,

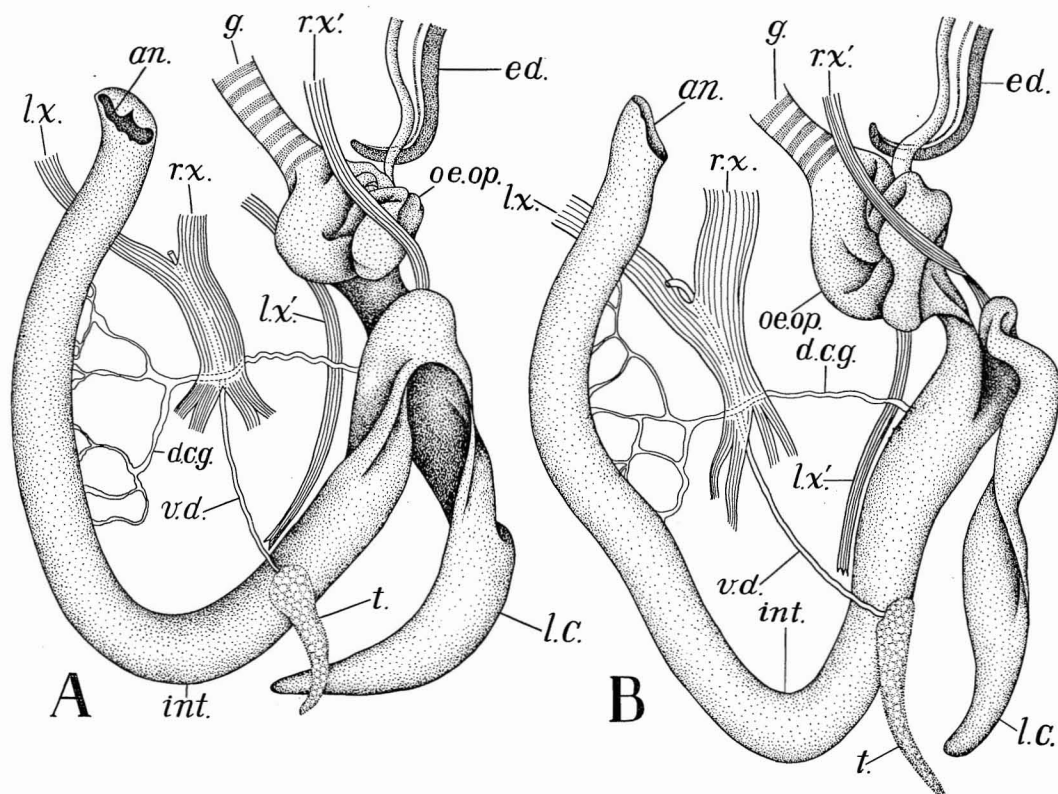


FIG. 6. A, *Cyclosalpa strongyleteron* Berner 1955, visceral region of the aggregate form, sinistral individual, from the right side. B, Same for dextral individual.

Abbreviations: *an.*, anus; *d.c.g.*, duct of the circum-intestinal gland; *ed.*, endostyle; *em.*, embryo; *g.*, gill; *ht.*, heart; *int.*, intestine; *l.c.*, left blind sac; *l.o.*, luminous organ; *l.x.*, left dorsal visceral muscle; *l.x'*, left ventral visceral muscle; *oe.*, oesophagus; *oe.op.*, right blind sac; *r.c.*, right dorsal visceral muscle; *r.x.*, right dorsal visceral muscle; *r.x'*, right ventral visceral muscle; *t.*, testis; *v.d.*, vas deferens.

crosses the duct of the circum-intestinal gland on the left side, and extends to near the base of the testis. The right visceral muscle (*r.x.*) extends along the left side of the left caecum to a point near its posterobasal portion.

In the sinistral individuals the small right caecum is completely missing. The left caecum turns across the alimentary tract on the right side as shown in Figure 6A. The duct of the circum-intestinal gland opens at the base of the left caecum. Other structures are the same as in the dextral individuals.

The asymmetries found in the relation between the large (left) caecum and the small, or absent, right caecum, the arrangement of

the testis, vas deferens, duct of the circum-intestinal gland and the visceral muscles, are all of a primary character. The twist of the left caecum, shown in the sinistral individuals, may be considered to have been brought about by enatiomorphism.

The structure of the intestinal loop of the aggregate form of *Cyclosalpa bakeri* Ritter 1905 (Fig. 8) closely resembles that of the aggregate form of *C. strongyleteron*. It is U-shaped, with the anus (*an.*) opening on the same level as the oesophageal opening (*oe. op.*). The right caecum is absent in both the dextral and the sinistral individuals. In the dextral individuals the left caecum (*l.c.*) is attached ven-



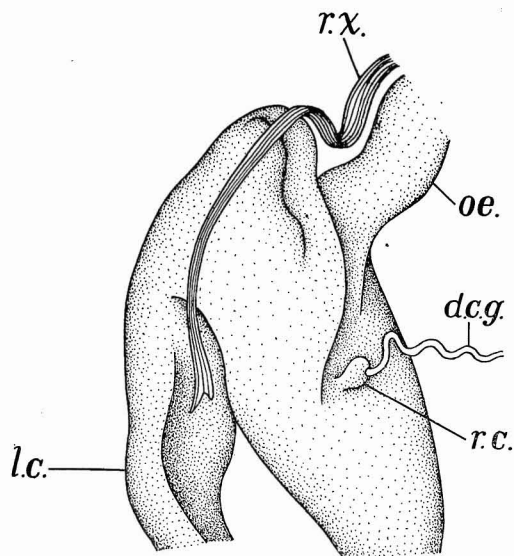


FIG. 7. *Cyclosalpa strongylenteron* Berner 1955, basal portion of left blind sac from left side.

trally about two thirds of the way down the lower branch of the intestinal loop. Its distal end reaches far beyond the posterior margin of the loop. In the sinistral individuals the caecum is twisted counterclockwise around the alimentary tract, as in the case of the sinistral individuals of *C. strongylenteron*. In the dextral individuals the duct of the circum-intestinal gland (*d.c.g.*) opens into the intestine on the left side at the level of the antero-basal portion of the caecum. In the sinistral individuals it opens into the base of the caecum itself.

The testis (*t.*) is held completely within a posterior protuberance projecting from the posterior portion of the intestinal loop. It is considerably longer than in *C. strongylenteron*. The vas deferens runs across the right side of the intestine, crosses the duct of the circum-intestinal gland on the left side, and opens into the atrial cavity at a position far anterior to the anus.

The right visceral muscle (*r.x.*) runs down the right side of the intestinal loop to its posterior margin. The left visceral muscle (*l.x.*) projects deep into the posterior protuberance

with the testis. In the dextral individuals the caecum is superficially placed in the median plane and asymmetry can be found only in the arrangement of the vas deferens and the difference in length of the visceral muscles. Such asymmetries are all of a primary nature.

If the structure of the visceral regions of the aggregate forms of *C. bakeri*, *C. strongylenteron*, and *C. virgula* are compared, we may be able to say to which *C. strongylenteron* is more closely related. On this basis it occupies an intermediate position between *C. virgula*, which has the small right caecum in both the dextral and sinistral individuals, and *C. bakeri*, in which the right caecum is lacking in both individuals. If, however, the musculature is phylogenetically more important than the structure of the visceral portion of the body, the above hypothesis is untenable.

During the examination of *C. bakeri* two

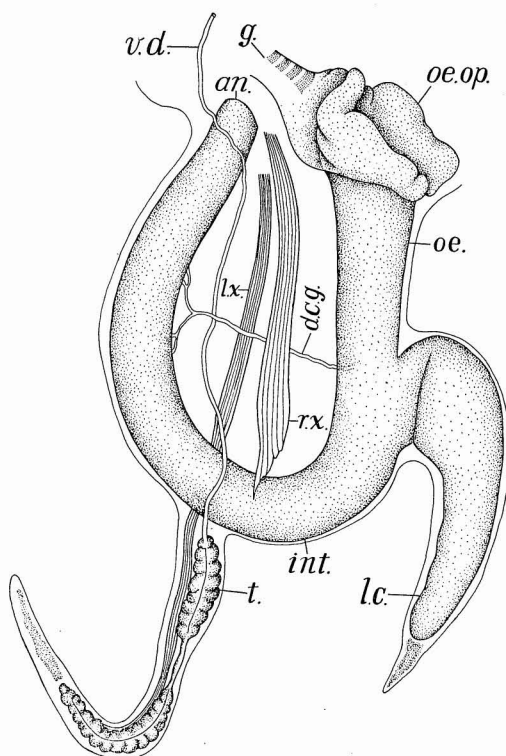


FIG. 8. *Cyclosalpa bakeri* Ritter 1905, visceral portion of dextral individual, from right side.

individuals of the aggregate generation were found which appeared to have light organs (Fig. 9). The organs were situated mid-laterally, one on each side of the body between muscles II and III. This is the first report of possible light organs in the aggregate generation of *C. bakeri*.

#### SUMMARY

Two new species in the genus *Doliolina*, *D. obscura* and *D. separata*, have been described. The genus *Doliolina* has been subdivided into two groups. The first is the *Doliolina* perfecta, in which all the muscle bands form complete loops, the second the *Doliolina* imperfecta, in which the seventh muscle band is interrupted ventrally.

The presence of fingerlike protuberances on the stomach of *Doliopsoides horizoni* have been described. Specimens of *Doliolina undulata* in which the undulations of the testis are much reduced are described. This straightening of the testis appears to be associated with early maturity.

*Doliolina intermedium* is reported from the central and tropical Pacific Ocean. The specimens differ from those previously described in having a slightly longer testis and in the nearness of the ovary to muscle VII.

The structure of the intestinal region of *C. strongyleteron* and *C. bakeri* are reviewed. On the basis of the structure of this region *C. strongyleteron* is placed in a position intermediate between *C. bakeri* and *C. virgula*.

The presence of what appear to be light organs in the aggregate generation of *C. bakeri* is noted.

#### REFERENCES

- BERNER, L. D. 1955. Two new pelagic tunicates from the eastern Pacific Ocean. *Pacific Sci.* 9(2): 247-253.  
 GARSTANG, W. 1933. Report on the Tunicata. Part I, Doliolida. *Brit. Antarctic (Terra*

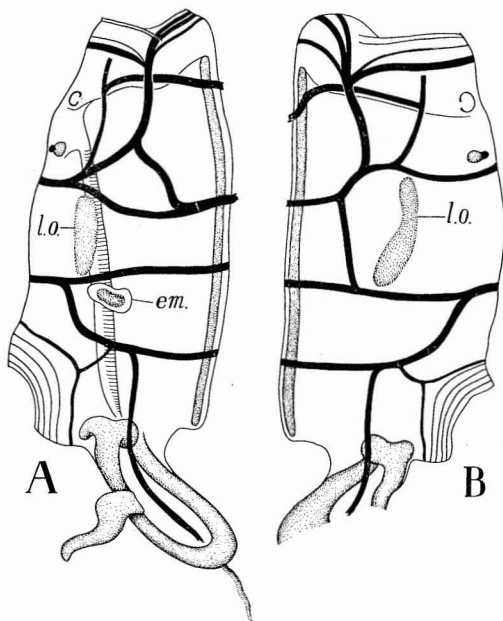


FIG. 9. *Cyclosalpa bakeri*, a 12 mm. aggregate form showing placement of luminous organ. A, from right side; B, from left side.

- Nova*) Exped. 1910, *Brit. Mus. Nat. Hist., Zool. Rpt.* 4 (6): 195-251.  
 HERDMAN, W. A. 1888. Report upon the Tunicata collected during the voyage of H.M.S. "Challenger," during the years 1873-76. III. Ascidiæ Salpiformes, Thaliacea, etc. IN *The Voyage of H.M.S. Challenger, Zoology* 27 (77): 1-163.  
 KROHN, A. D. 1852. Über die Gattung *Doliolum* und ihre Arten. *Arch. Naturgesch.* 28: 53-65.  
 NEUMANN, G. 1906. *Doliolum*. IN *Wiss. Ergeb. Deutsch. Tiefsee-Exped.* 12 (2): 93-243.  
 RITTER, W. E. 1905. The pelagic Tunicata of the San Diego region. *Calif. Univ., Pubs. Zool.* 2: 51-112.  
 TOKIOKA, T., and L. D. BERNER. 1958. Two new Doliolids from the eastern Pacific Ocean. *Pacific Sci.* 12(2):135-138.